



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/GB86/00711 (22) International Filing Date: 21 November 1986 (21.11.86) (31) Priority Application Number: 8528761 (32) Priority Date: 22 November 1985 (22.11.85) (33) Priority Country: GB		(74) Agent: GILL JENNINGS & EVERY; 53/64 Chancery Lane, London WC2A 1HN (GB). (81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US. Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(71) Applicant (for all designated States except US): CORAL SOCIEDADE BRASILEIRA DE PESQUISAS E DESENVOLVIMENTO LTDA. [BR/BR]; Edificio Icaquera, Avenida Brigadeiro Faria Lima 1620, 8º Andar, 01452 São Paulo, SP (BR). (72) Inventor; and (75) Inventor/Applicant (for US only) : STARKIE, Selby, John [GB/GB]; Riverside Cottages, Clayhithe, Horningsea, Cambridgeshire CB5 9JB (GB).			
(54) Title: ENZYME-COUPLED ANTIBODIES			
(57) Abstract <p>An enzyme-coupled antibody comprises an enzyme, e.g. coupled to a monoclonal antibody, which can catalyse reactions which result in the death of cells bearing antigenic sites with which the antibody can bind. Such products have therapeutic value.</p>			

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ENZYME-COUPLED ANTIBODIES

This invention relates to antibodies and their use in killing specific organisms or tissue cells which carry appropriate antigens. The invention is thus of potential value in therapy.

Antibodies bind specifically with appropriate antigens. However, the presence of specific antibodies in body fluids, in vivo, and their binding with antigens of organisms or tissue cells, are not necessarily sufficient to kill the organisms or tissue cell. The primary causes of killing are phagocytosis, macrophages, and lysis by complement. Antibodies bound to antigens on the organism or tissue cell often mediate opsonisation or the activation of complement. The role of antibodies in this respect is incompletely understood, but may be related to the class or subclass of the antibody, the nature and distribution of the antigen, the availability and activation of various kinds of phagocytic and accessory cells and the concentration and nature of the components and inhibitors of complement.

It is of potential value to make the killing of cells carrying specific antigens dependent only on the relative concentration of antibody (or modified antibody) to antigen, and independent of other effect or agents.

WO-A-8303679 discloses "polydomas", i.e. the product of the fusion of a hybridoma with a B-lymphocyte or another hybridoma. The polydomas produce a hybrid monoclonal antibody having dual specificity, e.g. one specificity directed against a target antigen and the other against a moiety which permits a diagnosis to be made or which delivers an agent lethal to the target antigen or associated tissue.

It is well known to couple enzymes to antibodies. For example, enzymes such as alkaline phosphatase and horseradish peroxidase have been coupled to antibodies,

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and the properties of the enzyme used in order to detect antibody-antigen binding. For the aim of detection, it is appropriate that the substrate of the enzyme should undergo a readily-detectable (e.g. colour) change in the presence of the enzyme.

Toxins such as ricin, gelonin or diphtheria toxin may be bound to antibodies. Such toxins are enzymes and result in the death of cells by degradation of components of the protein synthetic mechanisms within the cell. To achieve this effect, the complex of antibody and enzyme requires introduction into the cytoplasm of the cell, usually by a mechanism of the cell itself characteristic of a fluid cell membrane. In general, organisms with non-fluid cell walls do not internalise complex molecules of this kind, so that toxin-conjugated antibodies would not kill some organisms.

In an enzyme-coupled antibody according to the present invention, the enzyme has the ability to catalyse degradative or synthetic reactions which result in the death of the cell.

The present invention is based on the concept that an enzyme attached to an antibody is brought into close proximity with the cell when the antibody binds with the antigen on the cell. The substrate of the enzyme is a substance close to, or part of, the cell or the antigen, or the fluid around the cell or the antigen. The antibody need not have been produced from a polydome, but can retain a single specificity.

Examples of suitable enzymes are lipases, phospholipases, proteases and glycases, substrates for which might be lipids, phospholipids, proteins, sugars and glycoproteins in the cell walls. Glucose oxidase is an example of an enzyme which has a natural plasma substrate, glucose; one of the products of glucose

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oxidation is hydrogen peroxide which can kill cells by oxidation of components of the cell wall.

Many of the components of complement are enzymes. When some forms of antibody bind with some forms of antigen, the enzymatic components of complement are activated in a cascade, i.e. each activated component catalyses the activation of the next component. The final components, activated C789, constitute a phospholipase which digests parts of certain membranes, such as cell walls and basement membranes. The classical pathway of complement activation is initiated by the activation of the first components by antibody bound to an antigen, but not by a free antibody. By contrast, the concept of the present invention is that the enzyme is irreversibly bound to the antibody.

It will readily be appreciated that an enzyme-coupled antibody according to the invention can be used therapeutically. For example, in order to kill an organism or cell which has been detected in a subject, an enzyme selected to kill the cell can be coupled to an antibody, e.g. a monoclonal antibody, specific to an antigen of the organism or cell, and the coupled product administered to the subject. Coupling can be by any convenient, conventional method, e.g. the glutaraldehyde or the SPDP method. Administration may also be by any suitable conventional method, e.g. inoculation; for inoculation, a conventional composition may be formulated which comprises the product and a physiologically-acceptable excipient.

30 Example

A monoclonal antibody against Shigella as described in Example 1 of WO-A-8600646 is coupled, not with alkaline phosphatase as described in section G therein, but with glucose oxidase. Administration of the enzyme-coupled antibody into plasma containing the

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antigen (as determined by conventional procedures) leads
to (i) antibody-antigen reaction and thus the proximity
of enzyme and antigen-bearing cell, and (ii) the local
production of cell-toxic hydrogen peroxide by enzymatic
5 oxidation of glucose in the plasma.

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CLAIMS

1. An enzyme-coupled antibody, in which the enzyme is characterised by ability to catalyse reactions which result in the death of cells bearing antigenic sites with which the antibody can bind.
2. An enzyme-coupled antibody according to claim 1, in which the enzyme catalyses destruction of the cell wall.
3. An enzyme-coupled antibody according to claim 1, in which the substrate for the enzyme is present in plasma and the cells are destroyed by a product of the enzymatic reaction.
4. An enzyme-coupled antibody according to any preceding claim, in which the antibody is monoclonal.
5. An enzyme-coupled antibody according to any preceding claim, for therapeutic use.
6. A method for treating a subject hosting an antigen, which comprises administering to the subject an effective amount of an enzyme-coupled antibody, in which the antibody is specific for the antigen and the enzyme has the ability to cause destruction of cells bearing the antigen.
7. A method according to claim 6, in which the enzyme-coupled antibody is as defined in any of claims 2 to 4.

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INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 86/00711

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC ⁴ : A 61 K 39/395; A 61 K 47/00; C 07 K 15/00																				
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched ⁷</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 30%; border-bottom: 1px solid black;">Classification System</th> <th style="border-bottom: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="padding: 5px;">IPC ⁴</td> <td style="padding: 5px;">A 61 K; C 12 P</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁸</div>			Classification System	Classification Symbols	IPC ⁴	A 61 K; C 12 P														
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III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; border-bottom: 1px solid black;">Category ⁹</th> <th style="width: 60%; border-bottom: 1px solid black;">Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²</th> <th style="width: 30%; border-bottom: 1px solid black;">Relevant to Claim No. ¹³</th> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">Chemical Abstracts, volume 79, no. 21, 26 November 1973, (Columbus, Ohio, US), T.J. Sullivan et al.: "Specific killing of parasites by antibody-enzyme conjugates", see abstract no. 124533b, & Res. Commun. Chem. Pathol. Pharmacol. 1973, 6(2), 709-17 --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1,3</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">Biological Abstracts, volume 80, no. 6, 1985, R.B. Lal et al.: "Selective elimination of lymphocyte subpopulations by monoclonal antibody-enzyme conjugates" see page 438, abstract 49774, & J. Immunol. Methods 79(2): 307-318, May 1985 --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1,4,5</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="text-align: center; vertical-align: top; padding: 5px;">--</td> <td style="text-align: center; vertical-align: top; padding: 5px;">3</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">FR, A, 2543969 (KUREHA KAGAKU KOGYO K.K.) 12 October 1984 see claims 1,22,30,31,43 --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1,4,5</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">CA, A, 1168150 (GOVERNORS OF THE UNIVERSITY OF ALBERTA) 29 May 1984 ./.</td> <td></td> </tr> </table>			Category ⁹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	X	Chemical Abstracts, volume 79, no. 21, 26 November 1973, (Columbus, Ohio, US), T.J. Sullivan et al.: "Specific killing of parasites by antibody-enzyme conjugates", see abstract no. 124533b, & Res. Commun. Chem. Pathol. Pharmacol. 1973, 6(2), 709-17 --	1,3	Y	Biological Abstracts, volume 80, no. 6, 1985, R.B. Lal et al.: "Selective elimination of lymphocyte subpopulations by monoclonal antibody-enzyme conjugates" see page 438, abstract 49774, & J. Immunol. Methods 79(2): 307-318, May 1985 --	1,4,5	A	--	3	Y	FR, A, 2543969 (KUREHA KAGAKU KOGYO K.K.) 12 October 1984 see claims 1,22,30,31,43 --	1,4,5	Y	CA, A, 1168150 (GOVERNORS OF THE UNIVERSITY OF ALBERTA) 29 May 1984 ./.	
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>																				
IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">Date of the Actual Completion of the International Search</td> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">Date of Mailing of this International Search Report</td> </tr> <tr> <td style="text-align: center; padding: 5px;">27th February 1987</td> <td style="text-align: center; padding: 5px;">1- 2 APR 1987</td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px;">International Searching Authority</td> <td style="border-bottom: 1px solid black; padding: 5px;">Signature of Authorized Officer</td> </tr> <tr> <td style="text-align: center; padding: 5px;">EUROPEAN PATENT OFFICE</td> <td style="text-align: center; padding: 5px;">M. VAN MEC </td> </tr> </table>			Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	27th February 1987	1- 2 APR 1987	International Searching Authority	Signature of Authorized Officer	EUROPEAN PATENT OFFICE	M. VAN MEC										
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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

P,X	see claims 1,3,6,11 -- WO, A, 86/00646 (TECHNOLOGY LICENCE CO. LTD) 30 January 1986 see claims 1,41,42,62 cited in the application -----	1,5 1,4,5
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V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 6+7 because they relate to subject matter not required to be searched by this Authority, namely:
 See PCT Rule 39.1(iv):
 Methods for treatment of the human or animal body by means of surgery or therapy, as well as diagnostic methods.
2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This international Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/GB 86/00711 (SA 15350)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 10/03/87

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-A- 2543969	12/10/84	JP-A- 59187794	24/10/84
		GB-A- 2138444	24/10/84
		SE-A- 8401943	22/11/84
		DE-A- 3413339	11/04/85
		JP-A- 59188559	25/10/84
		JP-A- 59186925	23/10/84
CA-A- 1168150	29/05/84	None	
WO-A- 8600646	30/01/86	EP-A- 0189451	06/08/86
		JP-T- 61502632	13/11/86

For more details about this annex :
see Official Journal of the European Patent Office, No. 12/82